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HOST SPECIFICITY AND OVIPOSITION OF *UROPHORA SIRUNASEVA* (HERING) (DIPTERA: TEPHRITIDAE), A NATURAL ENEMY OF YELLOW STARTHISTLE

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Abstract.—*Urophora sirunaseva* (Hering) (Diptera: Tephritidae) is a natural enemy of yellow starthistle (*Centaurea solstitialis* L., Asteraceae) in its range from Greece eastwards. Females oviposit into host capitula, and lignified, unilocular galls are formed around the developing larvae in the receptacle. In laboratory studies, *U. sirunaseva* oviposited preferentially in closed, immature capitula with vertically oriented involucral spines. The fly posited an average of 136 eggs per female. The results of laboratory, no-choice, host-specificity tests of flies from northern Greece indicate a high degree of host specificity to yellow starthistle: *U. sirunaseva* reproduced on yellow starthistle, but not on closely related, North American native or commercially important species.

Key Words: Insecta, *Urophora, Centaurea,* biocontrol of weeds, host specificity, gall

Yellow starthistle (*Centaurea solstitialis* L., Asteraceae) is an Eurasian annual that is a major weed of rangelands and other environments in the western United States (Maddox and Mayfield 1985, Maddox et al. 1985, Roché and Roché 1988, Callihan et al. 1989). The weed's area of gross infestation is ca. 3.2 million ha in California (Maddox and Mayfield 1985), ca. 400,000 ha in Oregon (E. Coombs, pers. comm., 1992), ca. 81,000 ha in Idaho (Callihan et al. 1989), and ca. 54,000 ha in Washington (Roché and Roché 1988). Yellow starthistle is highly invasive and is still expanding its naturalized range; its area of infestation increased ca. 420% in California between 1965 and 1985 (Maddox and Mayfield 1985). The spiny capitula deter grazing by livestock, and ingestion of the plant by horses can lead to a fatal neurological disorder called nigropallidal encephalomalacia or "chewing disease" (Cordy 1978). Yellow starthistle is presently the target of a biological control research program in the United States (Turner and Fornasari in press).

Urophora sirunaseva (Hering) (Diptera: Tephritidae) is a natural enemy of yellow starthistle in its range from Greece eastwards. Females oviposit in yellow starthistle capitula, and lignified, unilocular galls are formed around developing larvae in the receptacle. The fly is bivoltine and overwinters as mature larvae in galls. The genus *Urophora* has been the subject of recent systematic studies (White and Clement 1987, White and Korneyev 1989). White and Clement (1987) showed that *U. sirunaseva* from Greece could complete development on yellow starthistle from the United States. Field-plot, host-specificity tests were carried out in northern Greece by Groppe et al. (1990) and Clement and Sobhian (1991). The laboratory host-specificity and oviposition studies reported here complement these field tests. The fly is established as a biological control agent for yellow starthis-

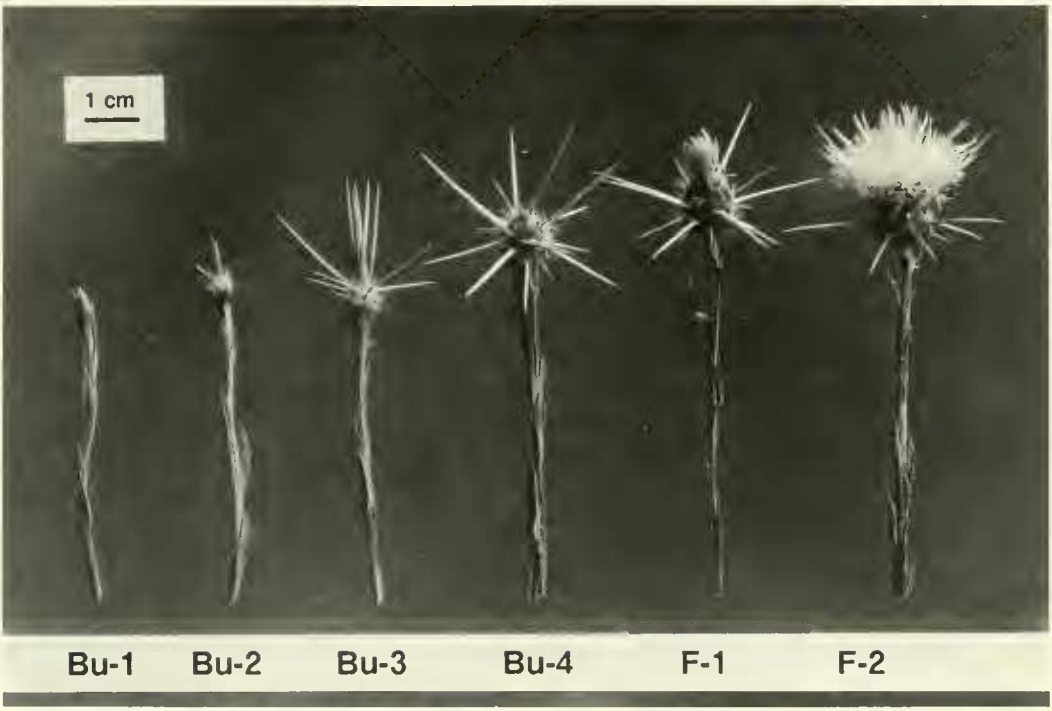

Fig. 1. Stages of capitulum development in yellow starthistle (after Maddox 1981).

tle in at least California, Oregon, and Washington (Turner et al. in press).

Materials and Methods

Yellow starthistle capitula from populations known to be infested by *U. sirunaseva* were collected in northern Greece and shipped to the biological control quarantine facility in Albany, California. The infested capitula were placed in sleeve cages for the emergence of adults, which were used (except where noted below) in the following studies conducted in the quarantine glasshouse facility. Photoperiod averaged ca. 14:10 (L:D) h (NOAA 1981), and average temperatures fluctuated from ca. 24°C (14 h) to ca. 19°C (10 h).

Oviposition studies.—An experiment was carried out to assess oviposition preference for the different stages of capitulum development in yellow starthistle as categorized by Maddox (1981) (Fig. 1): Bu-1 = bud at most only slightly expanded laterally, with spines barely evident; Bu-2 = bud moderately expanded laterally, spines greenish, vertical, and 1–2× the bud length; Bu-3 = bud globose, spines mostly vertical and 2–3× the bud length, some spines beginning to rotate away from vertical; Bu-4 = bud larger, spines straw-colored and all rotated away from vertical; F-1 = yellow flowers just visible and appressed but protruding from the involucre; F-2 = flowers fully exposed, collectively expanded beyond involucre immediately below. Thirty capitula of each of five different stages of capitulum development (stages F-1 and F-2 were lumped together; most were at the F-2 stage), from early, closed buds to flowering, were exposed to 20 recently emerged and mated females in a sleeve cage (ca. 0.07 m^3) with a wood frame, screening and cloth sleeves. The test capitula were set up as bouquets of capitula in a flask with water, with a plug of cotton where the stems containing one capitulum each emerged from the lip of each flask. Of a total of six flasks, each flask contained five capitula, at different develop-

Table 1. *Urophora sirunaseva* no-choice, host-specificity tests, Albany, California, 1988 and 1991.

Test Plant Species (Source)[1] (Year Tested)	No. Plants Tested	No. Capitula Tested	% Capitula with ≥1 Gall	x̄ Galls per Galled Capitulum
Centaurea solstitialis L. (ex California) (1988)	15	115	44.3	3.2
Centaurea solstitialis (ex California) (1991)	10	346	42.4	2.6
Centaurea solstitialis (ex Italy) (1988)	5	49	28.5	2.0
Centaurea americana Nuttall (ex Texas) (1988)	10	47	0	
Centaurea calcitrapa L. (ex California) (1991)	9	159	0	
Centaurea cyanus L. (1988)	15	131	0[2]	
Carthamus tinctoria L. var. S541 (1988)	15	47	0	
Carthamus tinctoria var. 4440 (1988)	15	80	0	
Cirsium ochrocentrum Gray (ex California) (1991)	10	27	0	

[1] Where collected from wild populations.
[2] Total of three non-viable, thin-walled galls were produced; these would not have been counted as galls in *C. solstitialis*.

mental stages (Bu-1, Bu-2, Bu-3, Bu-4, and F-1/F-2). The flies had free movement within the cage. This experiment was carried out under natural light for 48 h, 31 July to 2 August, 1989, at which time the flies were removed and the capitula dissected and examined for eggs. The number of eggs posited were tallied for each of these capitulum stages.

In 1991, a simple egg production study was carried out with ten newly emerged males and females placed together in a sleeve cage with bouquets of ten yellow starthistle capitula with a preponderance of vertically oriented spines (Bu-2 and Bu-3 stages). The study was carried out under natural light, and food was provided as streaks of honey on the cage. Every two days, the capitula were removed (and replaced with fresh capitula), dissected, and examined for eggs. The flies were first put into the cage with the bouquets of capitula on 30 July. All the females survived through 18 August; five had died by 19 August. The study ended 21 August, with the death of all females.

The size of eggs was measured in 1992 from 30 eggs removed from bouquets of closed capitula exposed to ten males and females. These flies had emerged from galled capitula, which were collected 19 June 1992 from an established *U. sirunaseva* population in Placer County, California. Freshly harvested eggs were measured through a stereomicroscope provided with an ocular graticule.

Host specificity.—No-choice, host-specificity experiments were carried out in 1 m^3 screen cages in 1988 and 1991. The test plant taxa consisted of economically important, native, or weedy relatives, all in the Cardueae: yellow starthistle, purple starthistle (*Centaurea calcitrapa* L.), cornflower (*Centaurea cyanus* L.), American basketflower (*Centaurea americana* Nuttall), two varieties of safflower (*Carthamus tinctorius* L. var. S541 and *C. tinctorius* var. 4440), and yellowspine thistle (*Cirsium ochrocentrum* Gray). Yellow starthistle, purple starthistle and cornflower are naturalized in North America. American basketflower and yellowspine thistle are native in the United States.

The screen cages enclosed 9–15 plants per species, each plant in a 15 cm pot. Each cage contained all the test plants of a plant species, thus one test plant species per cage ("no-choice" tests). Recently emerged male and female flies were placed in the cages with the test plants at the ratio of one male-female pair per test plant. Thus, for example, 15 yellow starthistle plants were tested in a cage with 15 male-female pairs of flies in 1988 (Table 1). The flies could move freely on and between plants, and all test plants had capitula at stages suitable for oviposition. Food was provided as honey streaked

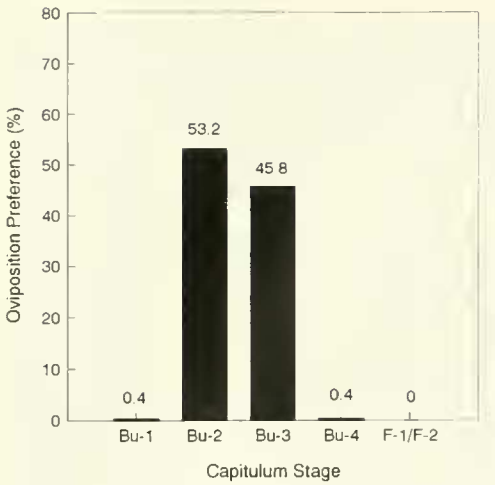

Fig. 2. *Urophora sirunaseva* oviposition preference by stage of capitulum development in yellow starthistle, percentage of total eggs posited (n = 203 eggs).

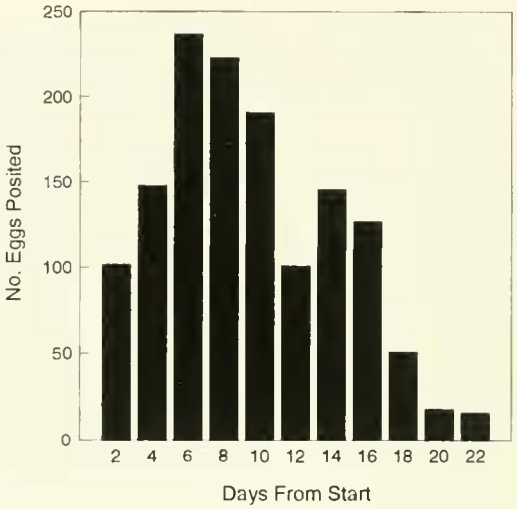

Fig. 3. Time course of oviposition by *Urophora sirunaseva* on yellow starthistle—number of eggs posited (Total = 1360 eggs) by 10 newly emerged females in the presence of males, in two-day intervals from start of test.

on strips of waxed paper suspended from the top of each cage. For each test, the flies were enclosed in cages with the test plants under natural light for three weeks, at which point most flies were dead. The 1988 tests were conducted from 6 July to 11 August, and in 1991 from 24 June to 19 July. At the end of the tests, all capitula suitable for oviposition during the test period were dissected and examined for the presence of galls or other damage. The galled capitula from the 1988 tests were kept in jelly cups until the following year to test for completion of development (adult emergence).

Results and Discussion

Oviposition studies.—Females oviposit from the tops of closed capitula, and generally place the fusiform eggs between flower buds. Posited eggs are oriented somewhat vertically, with their more rounded tip seated on the receptacle and the more sharply tapered tip directed away from the receptacle. The width (mean ± SEM) of the eggs was 144 ± 0.9 μm, while the length of the eggs was 865 ± 12 μm, thus the egg length is ca. 6× width.

In the capitulum preference study, 203 eggs were recovered from 37 test capitula. There was a strong preference for closed capitula with vertically oriented involucral spines (stages Bu-2 and Bu-3) (Fig. 2). The other capitulum-attacking insect species [the weevils *Bangasternus orientalis* (Capiomont), *Eustenopus villosus* (Boheman), and *Larinus curtus* Hochhut, and the tephritid fly *Chaetorellia australis* Hering] currently established for biological control of yellow starthistle oviposit on capitula at other stages of development (Clement 1990, Turner and Fornasari in press).

In the egg production study, 1360 eggs were produced by the ten females, for an average of 136 eggs per female. Figure 3 shows the time course of oviposition from the start of the test, shortly after adult emergence. Oviposition peaked by the sixth day, after which it gradually declined due to a decrease in egg production per female (rather than female mortality), as all females were still alive through the eighteenth day.

Host specificity.—In host-specificity tests, *U. sirunaseva* reproduced only on yellow starthistle, with galls formed on 44.3%

(1988) and 42.4% (1991) of the test capitula from California yellow starthistle (Table 1). Adult flies emerged from 89.7% of the galls from the 1988 tests. During the 1991 tests, next generation flies began emerging from galled capitula in the test cage before the capitula were removed for examination.

These results are congruous with the field host-specificity experiments carried out in Greece by Groppe et al. (1990), and Clement and Sobhian (1991). Clement and Sobhian (1991) obtained *U. sirunaseva* reproduction on yellow starthistle from Greece and the United States, but not on safflower, artichoke (*Cynara scolymus* L.) or *Cirsium creticum* (Lamarck) D'Urville. Groppe et al. (1990) obtained reproduction on yellow starthistle from Greece, but not on *Centaurea diffusa* Lamarck, *Centaurea maculosa* Lamarck, safflower, artichoke, *C. creticum*, or sunflower (*Helianthus annuus* L.). The only recorded field hosts of the fly are yellow starthistle and the closely related *Centaurea idaea* Boissier & Heldreich in Crete (White and Korneyev 1989).

For the galled capitula from the California yellow starthistle, the number (mean ± SEM) of galls per galled capitulum were 3.2 ± 0.3 in 1988 (n = 51 galled capitula, maximum = 9 galls) and 2.6 ± 0.1 in 1991 (n = 147 galled capitula, maximum = 8 galls). Figure 4 shows the freqency distribution of the number of galls per galled capitulum from the California yellow starthistle test in 1991. Though less than half of the test capitula were galled by *U. sirunaseva* (Table 1), ca. two-thirds of the galled capitula had two or more galls (Fig. 4). This is in contrast to the number of flies developing per capitulum by *Chaetorellia australis*, another natural enemy of yellow starthistle, where in similar host tests only one fly developed from 92.3% (in 1986) and 94.5% (in 1987) of the infested yellow starthistle capitula, and no more than two flies developed from any capitula (Maddox et al. 1990). This difference is related to different larval biologies. The larvae of *U. sirunaseva* are relatively stationary in galls, while those of *C. australis* move through capitula, tunneling through developing seeds.

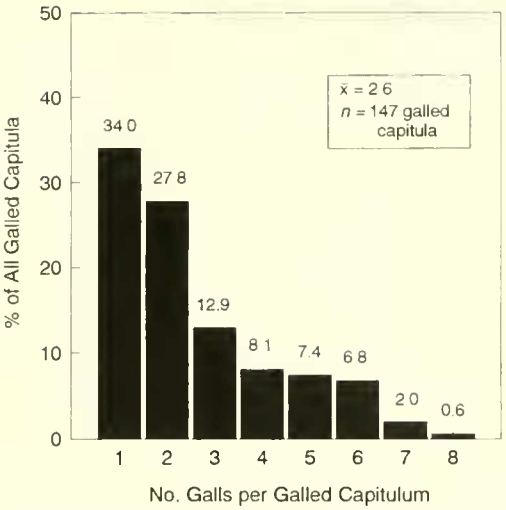

Fig. 4. Distribution frequency of number of *Urophora sirunaseva* galls per galled capitulum of yellow starthistle in a quarantine glasshouse test, 1991.

Although *U. sirunaseva* does not occur in Italy, in these tests it did reproduce on yellow starthistle from Italy (Table 1). In Italy, the closely related and biologically similar *U. jaculata* Rondani also attacks the capitula of yellow starthistle (White and Clement 1987, White and Korneyev 1989), but does not complete development on yellow starthistle from California, Idaho, or Washington (White and Clement 1987).

In conclusion, three different lines of evidence, (1) field host records (White and Korneyev 1989), (2) field, host-specificity experiments (Groppe et al. 1990, Clement and Sobhian 1991), and (3) laboratory, no-choice, host-specificity experiments, strongly indicate a very high level of host specificity and safety for *U. sirunaseva* as a biological control agent for yellow starthistle.

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